

# Aviremia 10 Years Postdiscontinuation of Antiretroviral Therapy Initiated During Primary Human Immunodeficiency Virus-1 Infection and Association With Gag-Specific T-Cell Responses

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**Combination antiretroviral therapy during primary human immunodeficiency virus-1 infection may enable long-term drug-free virological control in rare individuals. We describe a female who maintained aviremia and a normal CD4<sup>+</sup>/CD8<sup>+</sup> T cell ratio for 10 years after stopping therapy, despite a persistent viral reservoir. Cellular immune responses may have contributed to this outcome.**

**Keywords.** antiretroviral therapy; CD8 T cell; HIV-1 reservoir; primary HIV-1 infection; viral inhibition assay.

Achieving prolonged control of human immunodeficiency virus (HIV)-1 replication after antiretroviral therapy (ART)

interruption is one of the aims of present research efforts towards eradication, to alleviate HIV-1 burden, drug toxicity, and costs. Initiation of ART during primary HIV-1 infection (PHI) is associated with reduced size and diversity of viral reservoirs and enhanced immune preservation, relative to chronic infection. This is reflected in prolonged aviremia in a minority of early treated seroconverters who discontinue ART [1–3]. Elucidating the underlying mechanisms may facilitate the development of curative therapy. In this study, we describe the favorable outcome of a woman who initiated ART during a severe acute retroviral syndrome. She stopped therapy 6 years later and has since experienced a decade of apparent spontaneous control. We report the virological and immunological features of this unusual case.

## METHODS

### Quantification of the Human Immunodeficiency Virus-1 Reservoir

Written permission was obtained from the patient for reporting of her case. The patient was infected with a clade C virus. During 17 years of follow-up, HIV-1 viremia was quantified with several validated assays, which included non-B primers in accordance with laboratory and clinical practice at the time. Low copy viremia was determined as described in [Supplementary Methods](#). Quantification of cell-associated HIV-1 DNA in blood sampled during 2007–2013 was performed as described previously [4, 5]. Integrated HIV-1 DNA was determined in samples from 2013 onwards by Alu-HIV polymerase chain reaction (PCR), in addition to quantification of total and episomal 2-long terminal repeat (LTR) circles [6, 7] ([Supplementary Methods](#)).

### Human Leukocyte Antigen Typing

The patient's human leukocyte antigen (HLA) type was determined by ARMS-PCR using sequence-specific primers as follows: HLA A\*0101, \*3001, B\*4901, Cw\*0701, DRB1 \*0804, DQB1 \*0301 (homozygous at HLA-B, Cw, DR, and DQ loci).

### Assessment of Human Immunodeficiency Virus-1-Specific Cellular Immune Responses

T-cell responses to the entire HIV-1 proteome were assessed initially using pools of clade B 15-mer peptides overlapping by 11 amino acids and, subsequently, corresponding overlapping clade C 18-mers, (NIH AIDS Reagent Program, final concentration 2 µg/mL) in ex vivo interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) assays, as described previously [8]. Further details are given in [Supplementary Methods](#).

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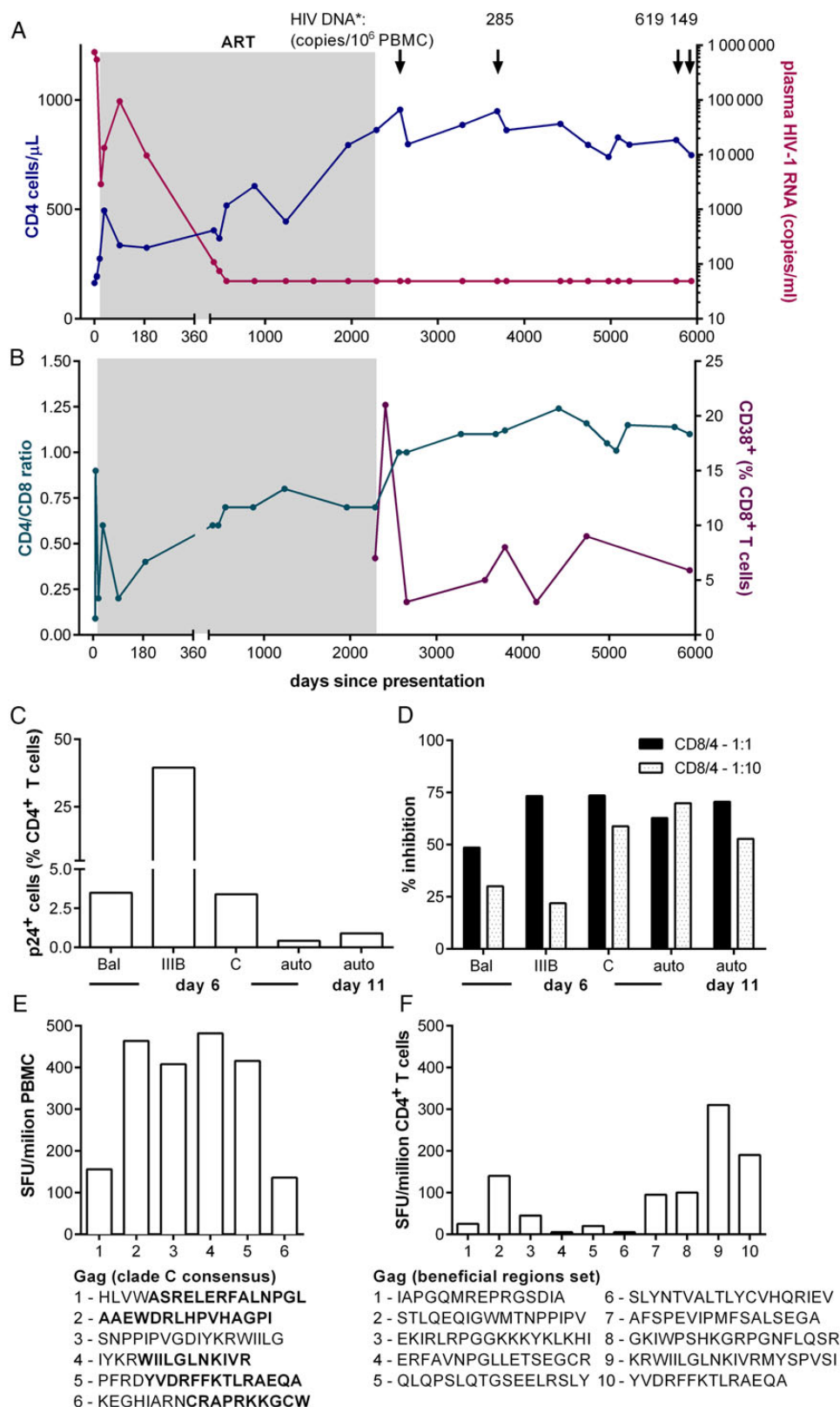
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**Figure 1.** CD4 cell count and plasma viral load changes (A), CD4/CD8 cell ratios, and percentage of activated (CD38<sup>+</sup>) CD8 T cells (B) during primary infection, antiretroviral therapy, and posttreatment discontinuation are shown. Antiretroviral therapy regimen: nucleoside reverse-transcriptase inhibitors zidovudine/lamivudine days 12–2121 and tenofovir/lamivudine days 2122–2289; protease inhibitors indinavir days 12–22, ritonavir (full dose) days 23–203, and ritonavir/saquinavir days 204–2289. The assays used to quantify human immunodeficiency virus (HIV)-1 RNA were as follows, in chronological order: Roche Amplicor HIV-1 Monitor version 1.0 assay with non-clade B primers added (first 2 samples in 1997); Roche COBAS Amplicor HIV-1 Monitor, replaced

Unfractionated and CD8<sup>+</sup>-depleted peripheral blood mononuclear cells (PBMCs) were also tested in IFN- $\gamma$  ELISPOT assays with pools of peptides representing “beneficial regions” that are preferentially targeted by individuals with reduced viral loads [9] (Supplementary Table 1).

Ex vivo CD8<sup>+</sup> T-cell viral inhibitory activity was determined using a viral inhibition assay as described previously [8]. Primary CD4<sup>+</sup> T cells were superinfected with laboratory-adapted or primary virus isolates (Programme EVA Centre for AIDS Reagents, National Institute for Biological Standards and Control) at a multiplicity of infection of 0.01: HIV-1BaL (CCR5-tropic, clade B), HIV-1IIIB (CXCR4-tropic, clade B), and HIV-1C (CCR5-tropic, clade C).

## RESULTS

### Case Report

A 23-year-old woman of Ethiopian origin presented in 1997 with a 3-week history of fever, sore throat, rash, lymphadenopathy, myalgia, and arthralgia. Her HIV-1 antibody test was positive with incomplete reactivity on HIV Western blot. She had tested HIV-1 seronegative in 1996. Three consecutive CD4<sup>+</sup> T-cell counts were <200 cells/mm<sup>3</sup>, and her viral load was initially >750 000 HIV-1 copies (c)/mL. The patient started zidovudine 250 mg twice daily, lamivudine 150 mg twice daily, and indinavir 800 mg 3 times a day. The latter was subsequently switched to ritonavir 600 mg twice daily because of intolerance. She remained viremic (up to 94 400 HIV-1 c/mL) while on treatment and required intensification (twice daily saquinavir 400 mg and ritonavir 400 mg), which was initiated in May 1998. Suppression of viremia <50 HIV-1 c/mL was not achieved until approximately 1 year later, but it was sustained for nearly 5 years thereafter, at which point ART was stopped at the patient's request. The pre-ART discontinuation CD4<sup>+</sup> T-cell count was 863 cells/mm<sup>3</sup> (30%), with a CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratio of 0.7. The patient has since remained asymptomatic with undetectable viremia, normal CD4<sup>+</sup> T-cell counts, and a CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratio >1 during 10 years of follow-up off ART (Figure 1). A comprehensive screen for antiviral agents by therapeutic drug monitoring on a blood sample from 2008 was negative (Delphic Laboratories Ltd, Merseybio Liverpool, UK). Furthermore, the

frequency of activated (CD38<sup>+</sup>) CD8<sup>+</sup> T cells, which was measured during 2004–2010, remained within normal limits (3%–21%) (Figure 1B). Human leukocyte antigen typing revealed that the patient did not express any HLA class I alleles associated with long-term control of HIV.

### Evidence of a Detectable Human Immunodeficiency Virus-1 Reservoir During Posttreatment Control

During the posttreatment period with aviremia, HIV-1 DNA was detected on 4 occasions: 2004 (not quantified), 2007, 2013, and 2014. Total HIV-1 DNA was 285, 619, and 149 copies/10<sup>6</sup> PBMCs in 2007, 2013, and 2014, respectively. At 15 years of aviremia (2014), integrated HIV-1 DNA and 2-LTR circles were 134.3 (95% confidence interval [CI], 56.5–304.4) and 3.9 (95% CI, 0–9.15) copies/10<sup>6</sup> PBMCs, respectively (Figure 1A). Viremia was undetectable by an ultrasensitive assay (<2 HIV-1 copies/mL).

### CD8<sup>+</sup> T Cells Sampled After 10 Years of Posttreatment Control Suppress Human Immunodeficiency Virus-1 Replication In Vitro

Ex vivo CD8<sup>+</sup> T cells were tested for their capacity to inhibit the replication of clade B and C HIV-1 isolates and of endogenous HIV-1 in autologous CD4<sup>+</sup> T cells in vitro. At a CD8<sup>+</sup>/CD4<sup>+</sup> ratio of 1:1, CD8<sup>+</sup> T cells demonstrated moderate antiviral activity against clade-matched (C, 73.5%) and mismatched (IIIB, 73.3%; BaL, 49%) virus isolates (Figure 1C and D). Efficient suppression of the clade C isolate was also observed at a lower CD8<sup>+</sup>/CD4<sup>+</sup> T-cell ratio (1:10%–59% vs 30% for IIIB and 22% for BaL). Antiviral activity against the patient's endogenous virus was equivalent to HIV-1C at both CD8<sup>+</sup>/CD4<sup>+</sup> T cell ratios (70.5% and 52.3% for 1:1 and 1:10 ratios, respectively) (Figure 1C and D).

### Broad CD4<sup>+</sup> T-Cell Responses Target Vulnerable Regions Within Gag

Interferon- $\gamma$ -secreting CD8<sup>+</sup> and CD4<sup>+</sup> T cells were predominantly targeted to 14 regions within HIV-1 Gag, Pol, and Nef (Figure 1E, Supplementary Figure 1A–C). T-cell responses were also targeted to 7 vulnerable (beneficial) regions within the clade B and C viral proteomes (Supplementary Table 1) and were predominantly CD4<sup>+</sup> T cell-mediated (Figure 1E and 1F, Supplementary Figure 1D).

*Figure 1 continued.* by version 1.5 (Ultrasensitive assay) in October 2004; Abbott LCx HIV-1 assay (January 2005–May 2005); Abbott Real-Time HIV-1 assay (May 2005 onwards). Antiretroviral agents included in therapeutic drug monitoring in 2008 were as follows: lamivudine, efavirenz, atazanavir, amprenavir, indinavir, nelfinavir, lopinavir, ritonavir, saquinavir, darunavir, tipranavir. (C) Susceptibility of the patient's CD4<sup>+</sup> T cells to superinfection with clade (B) and (C) virus isolates and to induction of endogenous HIV-1: frequencies of p24 Ag<sup>+</sup> CD4<sup>+</sup> T cells after in vitro activation of CD8-depleted peripheral blood mononuclear cells (PBMCs), followed by superinfection and culture for 6 days, or culture without superinfection (autologous virus) for up to 11 days. (D) Inhibition of replication of superinfecting or autologous viruses by patient's CD8<sup>+</sup> T cells after 6 days' coculture at CD8/CD4 cell ratios indicated. (E) Responses to individual clade C consensus Gag peptides identified in the peptide matrix assays were confirmed by interferon (IFN)- $\gamma$  enzyme-linked immunospot (ELISPOT) assays with unfractionated PBMCs. Negative control values have been subtracted. Some of these peptides overlapped “beneficial” regions within clade B and C Gag that were previously associated with virological control—shown in bold [9]. (F) CD4<sup>+</sup> T-cell responses to peptides representing beneficial regions within clade B/C Gag (listed in Supplementary Table 1) were determined in IFN- $\gamma$  ELISPOT assays with CD8-depleted PBMCs.

## DISCUSSION

This case shows that long-term control of viremia and normalization of immune parameters may follow discontinuation of prolonged ART initiated at PHI, even in the context of persistent viremia of nearly  $5 \log_{10}$  HIV-1 c/mL during initial ART and the absence of favorable HLA alleles. Such features would typically preclude long-term nonprogressor status [10].

Salgado et al and Sáez-Cirión et al have reported post-ART virological control after treatment during PHI, despite initial viremia of up to  $7 \log_{10}$  c/mL. The majority of these subjects had an inducible viral reservoir, indicating that they were infected with replication-competent viruses [2, 3]. We confirmed that our patient's CD4<sup>+</sup> T cells were susceptible to superinfection with R5- and X4-tropic viruses, with similar frequencies of infected cells to those we have observed following in vitro infection of healthy donor PBMCs [11]. Furthermore, we were able to detect outgrowth of endogenous HIV-1 in CD4<sup>+</sup> T cells after in vitro activation, albeit at a low level, indicating a persistent inducible reservoir. Although posttreatment controllers thus show some virological similarities with patients with spontaneous elite control of HIV-1, an intriguing difference is the lower activation status in the former [12]. Our patient also showed posttreatment control of immune activation: the frequency of CD38<sup>+</sup> CD8<sup>+</sup> T cells has remained within normal limits throughout follow up.

With regard to HIV-specific immune responses, this case is distinct from previously described posttreatment controllers [2, 3]. CD8<sup>+</sup> T-cell inhibition of both heterologous and autologous HIV-1 replication was detected at a low CD8<sup>+</sup>/CD4<sup>+</sup> cell ratio. Although these responses were less potent than was observed in HIV controllers (median 85%,  $n = 20$ ) [8, 13], they nevertheless surpassed that of chronic ART-treated individuals who were virologically suppressed for  $\geq 1$  year (median 24%,  $n = 42$ ; our unpublished observations). This result suggests that our patient's CD8<sup>+</sup> T-cell antiviral responses were likely to be a contributing factor to her virological control, rather than a consequence of it. The preserved and broad HIV-1-specific CD4<sup>+</sup> T-cell responses observed in this patient are also particularly surprising when taking into account the profound CD4<sup>+</sup> T-cell depletion observed before ART initiation. It is noteworthy that this patient had detectable CD4<sup>+</sup> T cell responses (polyfunctional and proliferative) in 2004 and 2008 (Supplementary Figures 2–4). Furthermore, we were able to show that CD4<sup>+</sup> T cells were targeted to multiple regions of vulnerability within Gag. Such responses have been associated with spontaneous control of viremia [9, 14]. It is not known whether this association is mediated by direct cytolytic mechanisms or indirectly, through provision of effective help to CD8<sup>+</sup> T cells. This deserves further exploration. We also considered the possibility that humoral responses contributed to this patient's posttreatment control, because broadly neutralizing antibodies may play a role in controlling viremia in rare cases [15]. However, we found

only low levels of neutralizing antibodies to heterologous virus in this case, when tested during the early period of aviremia in 2006–2007 (data not shown).

## CONCLUSIONS

In conclusion, we have identified an atypical case of posttreatment control, which may have been achieved through very early ART during PHI combined with effective cell-mediated immune responses. Our data suggest that strategies aiming to induce such immune responses during early ART might influence posttreatment virological control.

## Supplementary Material

Supplementary material is available online at Open Forum Infectious Diseases (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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**Potential conflicts of interest.** All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

## References

1. The Strategies for Management of Antiretroviral Therapy (SMART) Study Group. CD4<sup>+</sup> count-guided interruption of antiretroviral treatment. *N Engl J Med* **2006**; 355:2283–96.
2. Salgado M, Rabi SA, O'Connell K, et al. Prolonged control of replication-competent dual-tropic human immunodeficiency virus-1 following cessation of highly active antiretroviral therapy. *Retrovirology* **2011**; 8:97.
3. Sáez-Cirión A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI study. *PLoS Pathog* **2013**; 9:e1003211.
4. Avettand-Fenoë V, Chaix ML, Blanche S, et al. LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO 01). *J Med Virol* **2009**; 81:217–23.
5. Yerly S, Rutschmann OT, Opravil M, et al. Cell-associated HIV-1 RNA in blood as indicator of virus load in lymph nodes. *J Infect Dis* **1999**; 180:850–3.

6. De Spiegelaere W, Malatinkova E, Lynch L, et al. Quantification of integrated HIV DNA by repetitive-sampling Alu-HIV PCR on the basis of poisson statistics. *Clin Chem* **2014**; 60:886–95.
7. Malatinkova E, Kiselina M, Bonczkowski P, et al. Accurate quantification of episomal HIV-1 two-long terminal repeat circles by use of optimized DNA isolation and droplet digital PCR. *J Clin Microbiol* **2015**; 53:699–701.
8. Yang H, Wu H, Hancock G, et al. Antiviral inhibitory capacity of CD8+ T cells predicts the rate of CD4+ T-cell decline in HIV-1 infection. *J Infect Dis* **2012**; 206:552–61.
9. Mothe B, Llano A, Ibarrondo J, et al. Definition of the viral targets of protective HIV-1-specific T cell responses. *J Transl Med* **2011**; 9:208.
10. Madec Y, Boufassa F, Avettand-Fenoel V, et al. Early control of HIV-1 infection in long-term nonprogressors followed since diagnosis in the ANRS SEROCO/HEMOCO Cohort. *J Acquir Immune Defic Syndr* **2009**; 50:19–26.
11. Yang H, Yorke E, Hancock G, et al. Improved quantification of HIV-1-infected CD4+ T cells using an optimised method of intracellular HIV-1 gag p24 antigen detection. *J Immunol Methods* **2013**; 391:174–8.
12. Blankson JN, Bailey JR, Thayil S, et al. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. *J Virol* **2007**; 81:2508–18.
13. Hancock G, Yang H, Yorke E, et al. Identification of effective subdominant anti-HIV-1 CD8+ T cells within entire post-infection and post-vaccination immune responses. *PLoS Pathog* **2015**; 11:e1004658.
14. Ranasinghe S, Flanders M, Cutler S, et al. HIV-specific CD4 T cell responses to different viral proteins have discordant associations with viral load and clinical outcome. *J Virol* **2012**; 86:277–83.
15. Bonsignori M, Wiehe K, Grimm SK, et al. An autoreactive antibody from an SLE/HIV-1 individual broadly neutralizes HIV-1. *J Clin Invest* **2014**; 124:1835–43.